IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application No. 10/089,009

Applicant: Goldman et al.

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Examiner: Dong Jiang

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DECLARATION UNDER 37 C.F.R. § 1.132 OF THOMAS WALDMANN, M.D.

I, Thomas A. Waldmann, hereby declare that:

1. I am the Chief of the Metabolism Branch of the Center for Cancer Research at the National Cancer Institute (NCI), and a co-inventor of the subject patent application.

2. I have reviewed Colamonici et al., *J. Immunology*, *145*, 155-160 (1990) ("the Colamonici reference"), and understand that the Colamonici reference has been cited in the Office Action dated October 18, 2005, as allegedly anticipating the subject matter of the pending claims.

3. Using cross-linking and immunoprecipitation studies, the Colamonici reference discloses the identification of two polypeptides having a molecular weight of 37 kDa and 20 kDa, each of which reportedly associates with the p95-110 subunit of the IL-2 receptor (IL-2R) (see Colamonici reference at, e.g., page 159, second column).

4. The Colamonici reference further discloses the use of monoclonal antibodies anti-Tac and 7G7/B6 to immunoprecipitate IL-2R alpha and polypeptides associated

therewith, including the 37 kDa and 20 kDa polypeptides described above (see Colamonici et al. at, e.g., page 159, second column).

5. Work from my laboratory demonstrates that the polypeptides disclosed in Colamonici et al. are not the same polypeptides which are identified by the 5F7 monoclonal antibody as described in U.S. Patent Application No. 10/089,009.

6. Experimental results, found in Exhibit 1 (attached hereto), demonstrate that the 5F7 monoclonal antibody immunoprecipitates novel interleukin-2 receptor associated polypeptides (ILRAPs) compared to the ILRAPs immunoprecipitated with anti-Tac monoclonal antibody, as reflected by SDS-PAGE analysis. Exhibit 1 demonstrates an SDS-PAGE analysis using MT-1 cells, wherein the polypeptide bands immunoprecipitated by the 5F7 antibody are shown in lane 2 next to those polypeptide bands immunoprecipitated by the anti-Tac antibody in lane 3, run on the same gel. A labeled molecular weight marker is seen in lane 4, and lane 1 is a negative control. MT-1 cells are used in Colamonici et al. and are described as expressing a 37 kDa IL-2R binding peptide. Also, anti-Tac antibody and 7G7/B6 antibody, which detect CD25, are used in that study. In our study, the major polypeptide band immunoprecipitated with 5F7 antibody (lane 2) migrates to a different location than the polypeptide bands immunoprecipitated with anti-Tac antibody (lane 3), evidencing the difference between the 37 kDa polypeptide of Colamonici et al. relative to the novel 32-34 kDa polypeptide immunoprecipitated with 5F7 antibody. As these two peptides do not migrate in an equivalent fashion when immunoprecipitated and run side by side, they cannot be the same peptide.

7. A second experiment, found in Exhibit 2 (attached hereto), shows an SDS-PAGE analysis wherein lanes 2 and 3 are duplicates and show the polypeptides immunoprecipitated by the 5F7 antibody from surface labeled Kit 225 cells. The molecular wieghts of the two principle 5F7 polypeptide bands shown are determined using two independent sets of molecular weight markers as indicated on the exhibit. The labeled markers can be seen in lane 5, and unlabeled-stained markers are replicated in lane 1 directly from their position on the modified Western transfer. The migration results from the experiment reflected in Exhibit 2 estimate the size of the larger and denser polypeptide immunoprecipitated with 5F7 antibody to be between 31-36 kDa. Further, the smaller and less dense polypeptide band is estimated to be between 22-30 kDa. The resulting polypeptide sizes are different from those indicated in the Colamonici et al., and the differences are significant. In both cases the gel bands associated with polypeptide immunoprecipitated with 5F7 antibody are compared to specific molecular weight markers that differentiate them from

the polypeptide bands disclosed in Colamonici et al. Specifically, the major polypeptide band from this experiment ran below the 36 kDa molecular weight marker, differentiating it from the Colamonici et al. 37 kDa polypeptide band. Also, the minor polypeptide band from this experiment ran above the 22 kDa molecular weight marker, differentiating it from the Colamonici et al. 20 kDa polypeptide band. The results represented in Exhibit 2 provide further evidence that the IL-2 binding peptides described by Colamonici et al. are distinct from the peptides recognized by the 5F7 antibody in this patent application.

8. Exhibit 3 (attached hereto) further supports the view that the ILRAPs described in the subject patent application differ from those disclosed in Colamonici et al. The results presented in Exhibit 3 demonstrate the inability of the anti-Tac monoclonal antibody to remove the claimed ILRAPs from Kit 225 cellular lysate where both Tac and the claimed polypeptides are expressed. The comparative experiments were conducted on the same gel in order to remove any variability that could be caused by variations in individual gels. Lane 1 of the gel presented in Exhibit 3 is a negative control, and lane 4 contains a molecular weight marker with sizes indicated to the right of the gel. Lane 2 presents polypeptides immunoprecipitated by anti- 5F7 antibody from Kit 225 cell lysate, and lane 3 shows the presence of Tac protein immunoprecipitated by anti-Tac antibody from Kit 225 cell lysate. In this experiment, lysate from Kit 225 cells was pre-cleared multiple times using the anti-Tac antibody on an insoluble support, resulting in the removal of greater than 90% of the material recognized by the anti-Tac antibody. The remaining anti-Tac immunoprecipitate is shown in lane 6 and reflects the lack of remaining polypeptides recognized by anti-Tac antibody. However, when the same pre-cleared lysate is immunoprecipitated with anti-5F7 antibody, as shown in lane 8, the claimed polypeptides are still present with no variation in location and only a slight reduction in intensity relative to the initial levels presented in lane 2. Lane 2 reflects polypeptides immunoprecipitated by anti-5F7 antibody from non-pre-cleared, Kit 225 cell lysate, leading to the conclusion that the anti-Tac antibody of Colamonici et al. does not recognize the polypeptides identified by anti-5F7 antibody as evidenced by anti-Tac's inability to precipiatate the claimed polypeptides from the cellular lysate after the Tac protein was eliminated. Please note that lanes 5 and 7 are not involved in this experiment.

9. Exhibit 4 (attached hereto) is an experiment that shows the phenotypic expression of the novel 32-34 kDa ILRAP on the surface of Kit 225 cells and the lack of expression of the same polypeptide on MLA-144 cells as demonstrated by flow cytometry. In panel 1, Kit 225 cells are positive for expression of the novel 32-34 kDa ILRAP as shown by the green histogram when compared to the relative mean fluorescence intensity of a non-

relevant antibody overlaid in black. Such a result is expected because the novel 32-34 kDa ILRAP can be immunoprecipitated from the surface of Kit 225 cells as shown in Exhibit 2. Panel 2 of Exhibit 4 shows a flow cytometric phenotypic analysis of MLA-144 cells as negative for expression of the novel 32-34 kDa ILRAP. In contrast, Colamonici et al. indicates that MLA-144 cells are positive for the expression of the 37 kDa IL-2 binding polypeptide. *See* Colamonici et al. at Figure 5. However, the results presented herein do not find any reactivity of the 5F7 antibody with polypeptides expressed by MLA-144 cells, indicating that the 37 kDa IL-2 binding peptide described by Colamonici et al. on MLA-144 cannot be the same as the novel 32-34 kDa ILRAP, claimed in the patent application.

10. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: March 10 2006

Thomas A. Waldmann, M.D.